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What is Lyme Arthritis - also called Lyme Disease, & Chronic Lyme Arthritis ?

Lyme Arthritis is an epidemic, immune-mediated inflammatory disorder - usually begins with a characteristic skin lesion, ref. to as erythema chronicum migrans, - that may be followed weeks to months later by neurologic or cardiac abnormalities, migratory polyarthritis, intermittent attacks of oligoarticular arthritis, or chronic arthritis in the knees.

The endemic areas for this disorder include the northeastern coast of the U.S., particularly southern Connecticut, southern Rhode Island, Cape Cod, southern New York and Long Island, New Jersey, Delaware and Maryland. It has also been reported in Northern Wisconsin in northern California and Oregon.

Lyme Arthritis or Lyme disease got its name from the small community of Lyme, in eastern Connecticut where

Lyme arthritis is thought to be caused by an infectious agent transmitted by I. dammini.

By a characteristic expanding skin lesion, ECM, patients may often be identified 1-3 weeks after exposure and before the onset of arthritis.

When the skin lesion is present, most patients have circulating immune complexes. At that time, those with high serum IgM levels, cryoglobulins containing IgM, and low IgG levels are the ones at risk of developing arthritis within months. Those with high IgG usually do not.

-the name - Erythema chronicum migrans.

ECM appears about 1-3 weeks after tick-bite as an erythematous macule or papule. The borders of this lesion then expand to form a red ring as great as 20 to 30 cm in diameter, with central clearing. Occasionally secondary rings may form within the original one (erythema multiforme), sometimes expansion of the ring may not be accompanied by central clearing. The lesion which often itches, pricklies, or burns may be accompanied by fever, headache, vomiting, fatigue, and regional adenopathy.

It is of interest to note that the veterinary literature on European Lyme disease or ECM does not report or refer to arthritis - there is one possible exception - a report describing pain in a patient's ankle a few weeks after appearance of the ECM.

It also should be noted that the first case of ECM in the United States was reported in 1970, from the town of Redford in north central Wisconsin - so the

Even

A second passage to a third proved successful and produced the typical ECM rmp - two weeks after inoculation!

Of particular interest to us - as well as to the studies currently done at CDC in Atlanta - is a 1948 paper by Lenhoff (Lenhoff C, 1948 - spirochetes in aetiologically obscure diseases Acta Derm Venereol Stockh 28: 295-324)

who reported on the presence of spirochetes in the ECM lesion. However, subsequent studies by others using darkfield and phase contrast microscopy failed to confirm Lenhoff's claim.

In 1962, the French mycologists Degos, Tewraine and Mouret reviewed the clinical histories of 7 ECM cases that occurred in France between 1958 and 1961. They submitted the sera of their patients to Paul Giroud (L'Inst. Pasteur, Paris) who examined them for antibodies to rickettsial antigens by his famous slide agglutination test. Giroud's results showed antibodies in titers up to 1:32 against R. prowazekii and up to 1:160 against R. mooseri and R. conorii in early

disease has been recognized before the outbreak in Connecticut.

What are the factors that suggest that Lyme arthritis and ECM are tick-borne?

(1) - the geographic distribution of ECM and of Lyme arthritis coincide with the distribution of ixodid ticks, namely
I. ricinus in European countries
I. dammini along the northeastern coast and in Wisconsin and
I. pacificus in California and Oregon,

In many instances patients remembered having been bitten by ticks and in some instances patients provided the ticks for identification. Thus history of tick-bites prior to onset of the disorders are frequent.

(2) In a study carried out along the Connecticut river in Connecticut it was found that the incidence of Lyme disease during 1977 was 2.8 cases per 1,000 residents on the east side of the river - whereas on the west side the incidence was only 0.1 cases per 1,000 residents. An accurate epical survey revealed that the

population of *I. dammini* was much greater on the east compared to the west side of the river.

(3) The occurrence of cases in the summer and early fall, in the US at least coincided with activity seasons of *I. dammini*

(4) The incubation period of 1 to 3 weeks is relatively constant in both ECM & Lyme arthritis —

stick of ticks

There are areas such as in Scandinavia that report ECM cases but have no ticks; in those ~~areas~~ areas a mosquito vector has been suspected.

Alan Barbour

The causative agent(s) of ECM and Lyme arthritis are still unknown. Some investigators postulated a reaction to the tick bite — however the interval from the tick to the lesion — esp to Lymphocytoma seems too long for an allergic reaction to an ixodes antigen. The most widely held view is that an infectious agent is involved: in fact German investigators succeeded to demonstrate the lesion among themselves by means of the lesion from the edge of the lesion taking smear from the edge of the lesion (Binder F., Doepfmer, R., Horstein O., 1958, Klin Wochschr. 33: 727-728)

Let us now briefly discuss the biology of this unnamed tick vector and refer to observations made by Carey, Kinsley and Main, from the Department of Epidemiology and Public Health, Yale University School of Medicine at New Haven Conn. J. Med. Entomol. vol 17: 89-99, 1980 who studied this tick within the Lyme arthritis areas of southern Connecticut.

I. dammini like the other members of the Ixodes ricinus complex — has 3 developmental stages: the larva, the nymph, and the adult. Larval and nymphal ticks require a blood meal to reach the nymphal and adult stage, respectively. A blood meal is also required by the mated female tick before oviposition of eggs occurs. Male ticks do not ingest blood.

In 1978, according to Carey and associates, larval I. dammini were most abundant in the late summer and adults in the spring and fall.

I. dammini was found to parasitize a large variety of mammals — with the principle small host for larvae

and nymphs being the white-footed mouse (Peromyscus leucopus) and the Eastern chipmunk (Tamias striatus). The principal host for the adult was the white tailed deer. The immature stages of I. dammini most also eat dogs, and - dog may not be important host for all stages of I. dammini (Piesman et al. 1979, J. Med. Entomol. 15: 537-540)

unsuccessful. One should be aware that they have tested hundreds of patient sera for antibodies to tick-borne viral - other arboviral and bacterial agents with uniformly negative results

In 1988, Bob Philip and I tested a large series of Dr. Steere's sera by indirect immunofluorescence and microagglutination, respectively, against all available rickettsial antigens. The data were interpreted as being against a rickettsial etiology of Lyme disease.

Similarly, sera collected by Dr Claes Kindmark from ECM patients in Sweden had no significant titers when tested against the European members of the spotted fever group.

Last September, I unexpectedly received a shipment of adult I. dammini from my colleague, Dr. Jaye Banach from the New York Department of Health at Stony Brook. For the past few years Jaye and I have collaborated on NIH and Lyme Disease Foundation grants. He, more recently than Burgdorfer, established the role of I. dammini as a vector of Babesia microti, the

causative agent of human Cutaneous in that area. Attached to the tick vial was a pencil-written note saying, "Collected from an area where recent cases of Lyme arthritis have occurred. More ticks to follow, if you want to look at them - if not - don't. Jaye.

Yes, we were interested in these ticks, particularly since previous examination of I. dammini from that area yielded specimens infected with spotted fever rickettsiae. Attempts to isolate however, had failed.

Thus the 21 ♂♂ and 23 ♀♀ were subjected to the hemolymph test. Although they were negative for rickettsiae, two females contained in their hemolymph masses (clumps) of a microfilaria.

✓✓✓✓✓✓ slides.

Although I am not a helminthologist a review of the literature on zoonotic filarial infections of North America suggests that the "least" in question is a Dirofilaria.

Our slides have been forwarded for identification to the Department of Tropical Medicine and Public Health at the Tulane University.

until we receive final identification

~~In the meantime~~ let us realize that Dirofilaria is a nematode found in carnivores throughout the world mosquitoes and fleas are incriminated as the usual vector.

In the U.S. veterinarians see Dirofilaria as heartworms in dogs and subcutaneous infections in raccoons. About 50 human cases have so far been reported - most of these extremely rare - from New York, Long Island, Vermont, Massachusetts (Cape Cod), Florida and Wisconsin - i.e. within the distributional area of D. immitis

Could it be that this lack rather than magnitude of fleas in the vector to man?

After discovering the microfilariae, we decided to take a closer look at the remaining live ticks. Each tick was dissected for the preparation and examination of mean gut tissues including midgut salivary glands, Malpighian tubules, ganglion and genital systems.

Our first attention was directed towards a characterization of the tick's symbiotes.

Symbiotes as you must know are associated with every species of tick and occur in every tick specimen where they are usually limited to certain tissues such as the ovary, malpighian tubules and certain parts of the midgut. Their physiologic function and purpose are as yet unknown - but to the best of my knowledge they have ~~no~~ never been linked to disease of a tick's natural or accidental host. Because of their absence from the tissues of the salivary glands, their transmission by tick can be ruled out.

The other rickettsia-like symbiotes occur either free in the cytoplasm or

as colonies in membrane-bound vacuoles.

√√√ The symbiota of *T. dammini* - as illustrated in the next slides - are readily recognized as largely pleomorphic rickettsia-like organisms ranging from coccal to threadlike. They occur both in the cytoplasm where they produce massive infestations especially in the ovarial tissues.

In their fine structure they greatly differ from the symbiota of *Dermacentor*, for instance, in that

√ a) they are not membrane-bound

√ b) there is no differentiation of their cytoplasm, and

c) they also not exhibit a multilamellar cell wall.

They resemble rickettsiae in their gram appearance and presence of a typical slime layer but differ from pathogenic rickettsiae by the absence of a microcapsular layer and the fact that the outer and inner leaflets of the cell wall are of the same thickness (2-3 nm).

Presently we are in the process of isolating these symbiotes on tissue cultures for immunologic and immunocytochemical characterization.

The results of preliminary FA staining suggest that the T. dammini symbiotes have no antigenic relationship to the symbiotes of Dermacentor tick for instance or to the spotted fever or typhus group rickettsiae.

While examining the tissue smears of a series of 5 T. dammini ♀♀ I encountered yet another agent in the midgut of the second ♀ of that particular series — there it is - TT Spirochete!

To make certain that these organisms were alive, we prepared fresh preparations of midgut from the remains of the same tick and examined them under darkfield. Although many spirochetes were inactive, few exhibited the typical movement of BORRELIA. I shared this information with Jorge Benach who immediately

went with the field to collect and furnish additional ticks — and also with Alan Barbour who is collaborating with Dr. Hanner on the antigenic makeup of the relapsing fever agent, Borrelia hermsii.

As of today I have dissected 122 I. dammini (25♀♀ & 97♂♂) from the collection site in Long Island — 76 (12♀♀ & 64♂♂) were positive for spirochetes!

Interestingly these organisms appear to be present only in the ticks' digestive tract, particularly in the midgut where they are associated — often in clumps — with the microvillar brush border of the gut epithelium. Spirochetes have never been observed in the hemolymph or in any other tissue of the ticks.

That we are dealing here with a Borrelia became apparent also from the following slide that illustrates the fine structure of this microorganism.

The first - two slides are
longitudinal sections showing the
flagellum which consists of 6 to 8
fibrils (in the average) and
which forms an integral part
of the outer membrane. This is
better seen in cross sections
that also illustrate the plasma-
membrane (7-10 nm) surround-
ing a mottled cytoplasm with
randomly distributed ribosomes.

The diameter of these organisms
varies from .15 to .25 µ.

Of particular interest - and as
yet unsolved - is the "blebbing"
phenomenon - detectable even
by conventional light and
darkfield microscopy
(a bleb or aneurism
along the spirochete - as seen
on previous slide).

Electron microscopy reveals that
these blebs represent a bulging
or aneurism of the outer cell
membrane - forming a bleb
that may contain numerous
rather large granules. This
phenomenon has been the subject

of many previous investigations by spirochetologists and has been interpreted as a "sporulation phase" in the developmental cycle of Borrelia - that usually multiply by transverse fission.

However, there are other investigators such as Pillot & Ryter (Ann. Institut Pasteur, Oct. 107, 1964) who state "spherical forms are only a degeneration product of the helicoidal elements: they do not constitute resistance forms nor elements of an obligatory cycle.

I am certain that we will address ourselves to this question in the near future, for Alan has been successful in isolating and maintaining in Kelly's Medium the spirochete from a pool of tick midguts.

I should add here that inoculation of infected midgut suspensions and of heavily positive cultures into suckling and 21-day-old mice, also BALB/c RML white mice,

mice, meadow voles, guinea pigs, and rabbits did not result in detectable spirochetemias or illness.

√√ Finally, there was yet another agent (in many tick smears) that I first considered to be of protozoan nature —

show slide -

Does anyone who has ever heard of her work - recognize it?

Well, we had no difficulty in isolating this as Vero cell lysis - where we recognized it as the developmental stage of a yeast-originating from the exoskeleton of the ticks.

Thus my friends, we are looking at at least 4 parasitic or microbial agents that are associated with *I. dammini* from a highly endemic area of Lyme disease.

Without the knowledge that Penicillin is highly effective in treating ECM and

Lyme disease, one could speculate that each of these agents could be involved in the etiology of these disorders. Parasitism, tenacity, however, make the ixodesthia-like symbiotes and particularly the spirochete prime targets for further investigations.

Such investigations were initiated a few days ago with sera collected by Jorge Benach of Lyme disease patients from Long Island.

I am delighted to report that indirect fluorescence microscopy on either cultured or tick-associated spirochetes gave very sharp and specific staining reactions but was consistently negative when applied to the ixodesthialike symbiotes.

These findings - as far as the spirochetes are concerned at least - were confirmed also by Alan Weber who applied the Western Blot Technique to show the relationship between the patients' antibodies and the spirochetal antigens.